Review Article

Therapeutic Human Papillomavirus Vaccines: A Review

Vikrant C. Sangar*, Sweta T. Kothari, Abhay S. Chowdhary
Department of Virology and Immunology, Haffkine Institute for Training, Research and Testing, Mumbai, India.
***Corresponding author's E-mail:** vikrant.sangar701@gmail.com

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ABSTRACT

Human Papillomavirus (HPV) is the most common sexually transmitted disease which can infect different parts of the body including uterus. Cervical cancer is the second most common cancer among women worldwide. The HPV-related disease appears to be increasing but treatment is limited, difficult and insufficient with high relapse rate. This growth in disease burden had made the development of two an effective prophylactic Human Papillomavirus vaccines namely Gardasil® - quadrivalent (Merck) and Cervarix™ - bivalent (GlaxoSmithKline). These vaccines are widely marketed internationally. However, pre-existing HPV infection is a highly prevalent in public health of developed and developing countries. According to published literature, antigen-specific T cell-mediated immunity is required for treatment of established HPV infection. Therefore, it is an important to develop vaccines that induce cell-mediated immune responses which are specific for early viral proteins in order to effect regression of established HPV associated lesions and malignant tumours. The aim of therapeutic vaccine is to eliminate pre-existing lesions or cancer cells. As a result E1, E2, E5, E6 and E7 could be best candidate targets for therapeutic vaccine antigens. Research currently focuses on the development of various forms of HPV vaccines such as live-vector vaccines, peptide vaccines, protein vaccines, nucleic acid vaccines, cell-based vaccines, edible vaccines.

Keywords: Therapeutic Human Papillomavirus vaccines, Live-vector vaccines, Peptide vaccines, protein vaccine, nucleic acid vaccines, cell-based vaccines, edible vaccines.

INTRODUCTION

Human papillomaviruses (HPVs) are the primary etiologic agents of cervical cancer. Thus, cervical cancer and other HPV associated malignancies might be prevented by HPV vaccines. Currently, two HPV L1 VLP vaccines namely Gardasil® - quadrivalent (Merck) and Cervarix™ - bivalent (GlaxoSmithKline) are widely marketed internationally and these HPV vaccines are commercially available.[1] However, pre-existing HPV infection is highly prevalent in public health of developed and developing countries. According to published literature, antigen-specific T cell- mediated immunity is required for treatment of established HPV infection.[2] Therefore; it is an important to develop vaccines that induce cell-mediated immune responses which are specific for early viral proteins in order to effect regression of established HPV associated lesions and malignant tumours. The aim of therapeutic vaccine is to eliminate pre-existing lesions or cancer cells. As a result E1, E2, E5, E6 and E7 could be best candidate targets for therapeutic vaccine antigens. Most HPV therapeutic HPV vaccines target carcinoma-associated HPV proteins, particularly E6 and E7 because these two proteins are consistently expressed in most cervical cancer and their precursor lesions but absent in normal tissue. [3,4]

TYPES OF THERAPEUTIC VACCINES

Researchers are currently focusing on the development of various forms of HPV vaccines such as live-vector vaccines, peptide vaccines, protein vaccines, nucleic acid vaccines, cell-based vaccines, edible vaccines.[5]

1. Live-Vector Vaccines

Live-vector vaccines can be classified into: 1) viral vectors and 2) bacterial vectors. Live-vector vaccines are highly immunogenic because they can replicate within host cells and facilitate intercellular spread of antigen. Vaccination with live vectors causes increase in immunosuppressive factors in the host.[6]

Viral-Vector Vaccines

The live-vector-based vaccines include vaccinia virus, adenovirus, adeno-associated viruses have mostly been tested in preclinical models.

- ***Vaccinia virus vaccines***

Vaccinia virus (Vv) is a member of poxvirus family.[5] Vaccinia is considered as a convenient vehicle in the vaccination history because it has been extensively used in the eradication of smallpox.[7,8] In several pre-clinical studies, E6 and E7- specific immunotherapy using vaccinia vectors generate strong CTL activity and antitumor response.[9,10,11] Results of phase I/II clinical trials using TA-HPV indicates that after vaccination using recombinant Vv encoding HPV 16 and 18 E6/E7, patients with advanced cervical cancer, CIN III developed T cell immune response without any complications.[12] Vaccinia virus (Vv) vaccines have several advantages: 1) They offers high efficiency of infection and high levels of recombinant gene expression quickly; 2) They provides the opportunity to use this vector as safe vector for gene transfer into host antigen-presenting cells (APCs); 3) The virus is lytic, thus the

integration of vaccinia genome into host genome is extremely small.[5]

- ***Adenovirus vaccines***

Adeno-associated virus (AAV) is parovirus that is non-pathogenic in humans. Recombinant adenoviruses (AdV) are widely employed vectors to treat variety of illness. Recombinant AdV vectors encoding tumour- specific antigen P815A and β-gal can induce an antigen-specific CTL response and antitumor effect.[13,14] Adv vectors vaccines have several advantages: 1) They allow cloning of 8kb large gene; 2) They can be prepared easily in high titers and can transduce a wide range of cell types without integrating into the host genome.[5]

- ***Alphavirus vaccines***

Alphaviruses and their derivative vectors are attractive candidates for vaccine development.[5] Vaccination of mice with replication-defective Venezuelan equine encephalitis (VEE) virus replicon particle vector containing HPV 16 E7 RNA enhanced E7- specific CD8+ cell immune responses to eliminate established tumors.[15] These virus vaccines have several advantages: 1) They are highly efficient for introducing heterologous genes into target cells. 2) They hold promise for efficient delivery of antigen genes to target cells with low toxicity.[5]

Bacterial-Vector Vaccines

Bacterial vaccines are most inexpensive vaccines and recombinant bacteria have been successfully tested in animal models as vaccine candidates for HPV.

- ***Listeria vaccines***

Listeria monocytogenes is a gram-positive intracellular bacterium which acts as a promising bacterial vector in recombinant vaccine to prevent cancer. When *L. monocytogenes* is phagocytosed by macrophages, it is taken up in a phagosome. However, it escape into the cytoplasm of the macrophage by secreting a listeriolysin O, because of this property *Listeria monocytogenes* can deliver its antigens or carry foreign antigens into the both MHC-I and MHC-II pathways.[16] This bacterial vaccine has several advantages: 1) This vaccine induces strong cellular immunity. 2) This vaccine can be administered orally without loosing efficacy.[5]

- ***Other bacterial vaccines***

Other live attenuated bacteria like *Salmonella*, *Lactococcus lactis*, *Lactobacillus plantarum* and bacillus Calmette-Guérin are used for HPV vaccine preparation. Bacterial vector-based vaccines have been shown to be capable of eliciting strong E7-specific T-cell-mediated immune responses.[5,6]

2. Peptide Vaccines

Peptide vaccines are safe, easy to make at low cost and involve minimal regulatory issues during or after development. A major disadvantage is their very short half life *in vivo.* The identification and characterization of CTL epitopes for HPV has promoted the development of peptide vaccines against cervical cancer.[5,17] Peptide vaccines have potential clinical applicability. Immunization with a peptide derived from HPV 16 leads to the protection of mice against a lethal dose of HPV 16 transformed tumor cells. [18] When HPV associated cancer patients were injected with lapidated peptides derived from HPV 16 E7, CTL response were observed.[19] The potency of peptide vaccines can be enhanced by the use of adjuvants like Freund' adjuvant and Montanide ISA 51 adjuvant. The convenient use of peptide- based vaccines limited by MHC restriction and the necessity to define specific CTL epitopes. The preparation of peptide-based vaccines for use on a large scale is inefficient and laborious.[5] During the vaccine development process, selected HPV genes are inserted into yeast or another organism to produce large quantities of the chosen protein or peptides (which can be made synthetically also). However, it can be difficult to isolate the specific epitopes that elicit the desired immune response. Once the peptides are purified, they lack the microbial component which triggers the human immune system and therefore prompt weaker cytotoxic T- cell immune responses than whole pathogens. Multiple immunizations may be needed to produce long-lasting protective immunity.[20]

3. Protein Vaccines

To overcome the limitations of peptide vaccines, vaccine manufactures are looking at protein vaccines as option. Protein-based vaccines can present all possible epitopes of protein to immune system, thus bypassing all MHC restriction. In addition to that, protein-based vaccines offer certain safety advantages.[5]

One such example of a potential protein vaccine is TA-GW fusion protein that consists of HPV6- L2 fused to the E7 protein. It has been tested for the treatment of genital warts. Apart from this, Cantab Pharmaceuticals is researching on TH-GW/ pharmaccine and TA-CIN for HPV and to treat cervical dysplasia.[21] The TA-GW fusion protein vaccine consists of HPV6 L2 fused to E7 protein with alum adjuvant has been tested for clinical treatment of genital warts. In another study, TA- CIN fusion protein that consists of HPV16 L2/E6/E7 has been observed to induce E7 specific $CD8^+$ T cell immune response and prevent tumour formation in mice. The protein induced humoral and T- cell mediated immune response when tested in patients. The efficacy of HPV16 E7 peptide can be magnified when it is combined with adjuvants and fusion proteins. When tumour- bearing mice were immunized with HspE7, tumour regression was observed and protection from future development of the tumour was seen. Another group of researchers immunized women with HspE7 vaccine. After 4 months of administration, these women underwent loop electrosurgical excision procedure or cone biopsy. No evidence of CIN was found while 50% of the women reported tumour regression. None of the women

reported progressive cancer. HspE7 vaccine is currently in clinical trials at Stressgen Biotechnologies in Canada to treat asymptomatic HPV infections, HPV- associated cervical cancer and anal dysplasia.[5]

Protein vaccine is administered intramuscularly in multiple doses and these trials suggested that the vaccine was safe and immunogenic to clear the existing genital warts and reduced recurrence rates. The production process was transferred to SmithKline Beecham Biologicals (London, England), which reformulated the vaccine with a new, proprietary adjuvant (SBAS2) that heightens cell-mediated immune responses. The TA-CIN fusion protein consists of HPV 16 L2/E6/E7 with a novel adjuvant (NAX-57) is developed by NovaVax Inc. (Columbia, MD, USA) to induce E7-specific $CD8^+$ T-cell immune responses and tumor protection in mice.[20,22]

To develop therapeutic vaccines for HPV16 and HPV18, not only big pharmaceutical companies but also multiple academic institutions around the world including the National Cancer Institute (Bethesda, MD, USA), the Norris Cancer Center at the University of Southern California, the University of Leiden (Netherlands) and the University of Queensland (Woolloongabba, Australia) are sponsoring Phase I and II trials.[22,23,24]

4. Nucleic acid Vaccines

- ***Deoxyribose- Nucleic acid Vaccines***

Deoxyribose- Nucleic acid (DNA) vaccines are attractive for antigen-specific immunotherapy. These vaccines have several advantages: 1) naked DNA is safe, stable and relatively easy to manufacture. 2) These vaccines sustain the expression of antigen in cells for longer periods of time than RNA or protein vaccines.[5,25,26] The limitations of DNA vaccine are: 1) DNA may potentially integrate into the host genome which causes genomic instability. However, there is no evidence that shows that integration of DNA occurs in numerous organs or tissues. 2) DNA vaccines are poorly immunogenic because DNA lacks the intrinsic ability to amplify or spread from transfected cells to surrounding cells *in vivo*. Several strategies implemented by team of researchers to enhance the potency of DNA vaccines by: i) increasing the number of antigen-expressing DCs; ii) enhancing antigen processing and presentation in DCs and iii) improving DC interaction with T cells to augment T-cell mediated immune responses.[6]

- ***Ribose- Nucleic acid replicon Vaccines***

RNA replicon-based vaccines have more advantages for cancer vaccine development than any kind of vaccines because RNA replicons can replicate in a wide range of cell types and can be used to produce sustained levels of antigen expression in cells. However, RNA replicons are less stable than DNA. The use of RNA replicons is a relatively new and potentially interesting strategy for HPV vaccination. RNA-replicon-based vaccines can be used in patients repeatedly because many RNA replicon vectors do not contain viral structural genes so no infectious particles are produced.[5,6]

Studies demonstrated that potency of HPV 16 E7-specific self-replicating RNA vaccines can be applied by applying LAMP-1 targeting strategy, Mycobacterium tuberculosis HSP70 strategy or HSV1 VP22 strategy. DNA- based RNA replicons known as suicidal DNA share the advantages of both RNA replicons and naked DNA vaccines without the disadvantages of either form of vaccine.[27,28,29] Hsu et al., (2001) employed the DNA-launched RNA replicons for development of HPV vaccines and demonstrated significant E7- specific CTL activity and antitumor effects. Thus, RNA and DNA- launched RNA replicon vaccines are promising therapeutics options for treatment of HPV associated cervical cancer.[30,31]

5. Cell based Vaccines

Cell-based vaccines for cancer-immunotherapy are divided into two broad categories namely, dendritic cell-based vaccines and tumor cell-based vaccines.

- ***Dendritic cell-based vaccines***

Dendritic Cells (DCs) are most important Antigen Presenting Cells (APCs) in the immune system. When these cells are activated, they induce strong effector T-cell responses and memory. This concept is used to develop DC- based vaccines against different human malignancies. For generating therapeutic cervical cancer vaccines, greater understanding about the origin of DCs, their antigen-uptake mechanisms and signals to stimulate their migration and maturation into immunostimulatory APCs is required. It is postulated that DC- based vaccine includes, DCs pulsed with E6 and/ or E7 peptides/ proteins and DCs transduced with DNA, RNA or viral vectors encoding E6 and/ or E7. DC-based vaccines may be able to break peripheral tolerance.[22,31,33]

Presentation of peptides derived from HPV E6 and/ or E7 to immune system by DCs is a promising method of circumventing tumor -mediated immunosuppression. In several tumor models, treatment of tumors with peptide-pulsed DCs has resulted in tumour regression. According to a study, E7- pulsed DCs could induce both E7- specific $CD4^+$ T cell proliferative responses and strong $CD8^+$ CTL in patients with cervical cancer positive for HPV 16 and 18.[33] Gene- transduced DC- based vaccines are an attractive alternative to peptide- pulsed DC- based vaccines. The limitation to naked DNA transfer into DC is poor transfection efficiency using various physical methods. Tuting et al., (1997) described the use of a gun for particle- mediated transfer of genes encoding HPV16 E7 to generate DCs that express E7/MHC-I complexes. The vaccine not only generated an antigen-specific CTL response in vivo, it also promoted the rejection of an ordinarily lethal challenge with an HPV 16- transformed tumor cell line.[34] The potency of DC- based vaccines depend on the specific route of administration. Wang et al., (2000), transduced HPV 16 E7 gene into DC line using electroporation using E7- expression vector and

demonstrated that intramuscular administration of DC-E7 generated greatest antitumor immunity compared to subcutaneous and intravenous routes of administration.[5,35]

- ***Tumor cell-based vaccines***

The use of tumor cell-based vaccine is not suitable for the treatment of an early stage, pre-cancerous HPV-associated lesion but this vaccine is mostly reserved for the advanced HPV- associated cancer. Several HPV related tumor cell-based vaccines have been reported in preclinical model systems. Genes encoding co-stimulatory molecules such as cytokines are transduced in the tumour cells to enhance the immunogenicity required for T- cell activation and other anti- tumour effects. HPV vaccine consists of E7 expressing cervical cancer cells transduced with cytokine genes such as interleukins 2 and 12 and granulocyte- macrophage colony stimulating factor (GM-CSF), that can generate E7- specific CTL activities and protective antitumor immunity in immunized mice.[5]

6. Edible Vaccines

Plant biotechnology techniques have permitted scientists to insert the genes for human pathogens, such as HPV, hepatitis B, and cholera, into yeast or edible plants such as potatoes, carrots, and lettuce. These genetically engineered plants then produce and accumulate disease antigens in their tissues. Their fruits and vegetables may serve as an edible vaccine, since eating them can induce an immune response. Edible vaccines also do not require costly and complicated cold chains for distribution. Finally, it is far simpler and cheaper to give foodstuffs to vaccine recipients than injections, which require skilled professionals and strict attention to infection- preventive measures. These vaccines offer several practical advantages of special importance for developing countries, which may have difficulty paying for, storing, distributing, and administering traditional vaccines.[20]

CONCLUSION

The development of any human vaccine is highly challenging, complex and an expensive process. However, in order to speed up the control of cervical cancer and treat current infections, researchers at pharmaceutical companies, biotechnology firms around the world are actively developing candidates for both prophylactic and therapeutic HPV vaccines. Based on published studies we conclude that the various current approaches, including live-vector vaccines, peptide vaccines, protein vaccines, nucleic acid vaccines, cell-based vaccines, edible vaccines have their own strengths and weaknesses.

REFERENCES

1. Sangar VC, Ghongane BB, Prophylactic Human Papillomavirus Vaccines, International Journal of Pharma and Bio Sciences, 4(3), 2013, 124 – 133.
2. Benton C, Shahidullah H, Hunter JAA, Human papillomavirus in the immunosuppressed, In: Papillomavirus report, 1992, 23-26.
3. Stoler MH, Rhodes CR, Whitbeck A, Wolinsky SM, Chow LT, Broker TR, Human papillomavirus type 16 and 18 gene expression in cervical neoplasias, Human Pathol, 23, 1992, 117-128.
4. Vandepapeliere P, Barrasso R, Meijer CJ, Walboomers JM, Wettendorff M, Stanberry LR, Randomized controlled trial of an adjuvanted human papillomavirus (HPV) type 6 L2E7 vaccine: infection of external anogenital warts with multiple HPV types and failure of therapeutic vaccination, J Infect Dis, 192(12), 2005, 2099-2107.
5. Thomas ER, Shah KV, Cervical cancer: From Etiology to Prevention, *Kluver Academic Publishers,* 2004, 79-99.
6. Hung CF, Ma B, Monie A, Tsen SW, Wu TC, Therapeutic human papillomavirus vaccines: current clinical trials and future directions, Expert Opin Biol Ther, 8(4), 2008,: 421-439.
7. Shen Y, Nemunaitis J, Fighting Cancer with Vaccinia virus: Teaching New Tricks to an Old Dog, Molecular Therapy, 11(2), 2005, 180-195.
8. Behebani AM, The smallpox story: life and death of an old disease, *Microbiol Rev,* 47, 1983, 455-509.
9. Boursnell ME, Rutherford E, Hickling JE, Construction and characterization of a recombinant vaccinia virus expressing human papillomavirus proteins for immunotherapy of cervical cancer, Vaccine, 14, 1996, 1485-1494.
10. Gao L, Chain B, Sinclair C, Immune response to human papillomavirus type 16 E6 gene in a live vaccinia vector, Journal of General Virology, 75, 1994, 157-164.
11. Lin K-Y, Guarnieri FG, Staveley-O'carroll KF, Treatment of established tumours with a novel vaccine that enhances major histocompatibility class II presentation of tumor antigen, Cancer Research, 56, 1996, 21-26.
12. Borysiewics LK, Fiander A, Nimako M, A recombinant vaccinia virus encoding human papillomavirus type 16 and 18, E6 and E7 proteins in immunotherapy for cervical cancer, Lancet, 347, 1996, 1523-1527.
13. Chen PW, Wang M, Bornte V, Therapeutic antitumor response after immunization with a recombinant adenovirus encoding model tumor-associated antigen, J Immunol, 156, 1996, 224-231.
14. Warnier G, Duffour MT, Uyttenhove C, Induction of cytolytic T- cell response in mice with recombinant adenovirus coding for tumor antigen P815A, Int J of Cancer, 67: 1996, 303-310.
15. Velders MP, McElhiney S, Casetti MC, Eradication of established tumors by vaccination with Venezuelan equine encephalitis virus replicon particles delivering human papillomavirus 16 E7 RNA, Cancer Research, 61, 2001a, 7861-7867.
16. Villanueva MS, Sijts AJ, Pamer EG, listeriolysin is processed efficiently into an MHC class I- associated epitope in *Listeria monocytogenes*- infected cells, J Immunology, 155, 1995, 5227-5233.
17. Duggan-Keen MF, Brown MD, Stacey SN, Stern PL, Papillomavirus vaccine, Frontiers in Bioscience, 3, 1192-1208.

18. Feltkamp MC, Smith HL, Vierboom MP, Vaccination with cytotoxic T lymphocyte epitope- containing peptide protects against a tumor induced by human papillomavirus type 16- transformed cells, Eur J Immunology, 23, 1993, 2242-2249.

19. Steller MA, Gurski KJ, Murakami M, Cell medicated immunological response in cervical and vaginal cancer patients immunized with a lapidated epitope of human papillomavirus 16 E7, Clinical Cancer Research, 269, 1998, 1585-1588.

20. Kols A, Sherris J, HPV Vaccines: Promise and Challenges, PATH Bill & Melinda Gates Foundation, 2000, 1-45.

21. Hanissian J, Emerging HPV vaccines, Infectious Medicine, 14(4), 1997, 273-275.

22. Roden R, Ling M, Wu MD, Vaccination to Prevent and Treat Cervical Cancer, Human Pathology, 35(8), 2004, 971- 982.

23. Van Driel WJ, Vaccination with HPV16 peptides of patients with advanced cervical carcinoma: clinical evaluation of a phase I-II trial, European Journal of Cancer, 35(6), 1999, 946-952.

24. Muderspach L, Wilczynski S, Roman L, A phase I trial of a human papillomavirus (HPV) peptide vaccine for women with high-grade cervical and vulvar intraepithelial neoplasia who are HPV-16 positive, Clinical Cancer Research 6, 2000, 3406-3416.

25. Robinson HL, Torres CA. DNA vaccines, Seminars in Immunology, 9, 1997, 271-283.

26. Donnelly JJ, Ulmer JB, Shiver JW, DNA vaccines, Annual Reviews of Immunology, 15, 1997, 617-648.

27. Cheng WF, Hung CF, Chai CY, Enhancement of sindbis virus self-replicating RNA vaccine potency by linkage of herpes simplex virus type 1 VP22 protein to antigen, Journal of Virology, 75, 2001a, 2368-2376.

28. Cheng WF, Hung CF, Chai CY, Enhancement of sindbis virus self-replicating RNA vaccine potency by linking of Mycobacterium tuberculosis heat shock protein 70 gene to an antigen gene, Journal of Immunology, 166, 2001c, 6218-6226.

29. Cheng WF, Hung CF, Hsu KF, Enhancement of sindbis virus self-replicating RNA vaccine potency by targeting antigen to endosomal/ lysosomal compartments, Human Gene Therapy, 12, 2001d, 235-252.

30. Hsu KF, Hung KF, Cheng KF, Enhancement of suicidal DNA vaccine potency by linking Mycobacterium tuberculosis heat shock protein 70 to an antigen, Gene Therapy, 8, 2001, 376-383.

31. Melief CJ, Cancer immunotherapy by dendritic cells, Immunity, 29, 2008, 372-383.

32. Doan T, Herd KA, Lambert PF, Peripheral tolerance to human papillomavirus E7 oncoprotein occurs by cross-tolerization, is largely Th-2-independent, and is broken by dendritic cell immunization, Cancer Research, 60, 2000, 2810-2815.

33. Santin AD, Hermonat PL, Ravaggi A, Induction of human papillomavirus- specific CD4(+) and CD8(+) lymphocytes by E7-pulsed autologous dendritic cells in patients with human papillomavirus type 16- and 18-positive cervical cancer, Journal of Virology, 73, 1999, 5402-5410.

34. Tuting T, DeLeo AB, Lotze MT, Genetically modified bone marrow- derived dendritic cells expressing tumor-associated viral or "self" antigens induce antitumor immunity in vivo, European Journal of Immunology, 27, 1997, 2702-2707.

35. Wang TL, Ling M, Shih IM, Intramuscular administration of E7- transfected dendritic cells generates the most potent E7-specific anti-tumor immunity, Gene Therapy, 7, 2000, 726-733.

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